Individual behavioral responses of an intermediate host to a manipulative acanthocephalan parasite and effects of intra-specific parasitic competition

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Background: Parasites with complex life cycles depend on the ingestion of their intermediate host by the final host. To complete their life cycle successfully, parasites frequently manipulate the intermediate host`s behavior and appearance. Within host-parasite systems, there is considerable variation in terms of intermediate hosts behavioral response to infection.

Aim: Identify sources of parasite-induced variation in intermediate host's traits by focusing on intra- and inter-individual variation in behavioral responses to parasitic manipulation, taking infection intensity, and thus, parasitic competition into account.

Organism: The acanthocephalan parasite *Polymorphus minutus*, which alters the phototactic behavior and activity of its intermediate hosts, *Gammarus pulex*, thereby increasing the probability to get eaten by final hosts.

Methods: We repeatedly examined the behavior of individual *G. pulex* varying in intensity of infection with *P. minutus* from uninfected to multiple-infected. We analyzed phototactic responses and activity.

Results and conclusions: Individual gammarids differed in phototactic behavior and in activity patterns, with repeatability ranging from 20% to 50%. Infected gammarids showed greater between-individual variation than uninfected gammarids in phototaxis but not in activity. All uninfected gammarids were photophobic, whereas phototactic behavior of infected gammarids ranged from photophobia to photophilia. On average, multiple-infected gammarids were similarly photophobic as uninfected ones. Single-infected gammarids were less photophobic than uninfected and multiple-infected conspecifics. This suggests that intra-specific parasitic competition affects the manipulative abilities of parasites. Both groups of infected gammarids were on average less active than uninfected ones, and this effect was mainly driven by some infected individuals. In conclusion, behavioral variation of gammarids was caused by individual differences in responses to manipulation/infection on the one hand, and by the reduced manipulative capacities of parasites facing intra-specific competition on the other hand.

Introduction

Parasites with a complex life cycle mature in an intermediate host species, but reproduce sexually in a different, final host species (Schmid-Hempel, 2011). In order to achieve the host change, it is often necessary that the intermediate host is ingested by the parasite's final host (*trophic transmission*, Lafferty, 1999). This creates a strong selective pressure on the parasite to increase the probability that its intermediate host is eaten by the final host (Moore, 2002). While there are convincing examples that parasites manipulate the intermediate host`s behavior and appearance to successfully complete their life-cycle in some host-parasite systems (Moore 1983; Poulin, 1999; 2010; Bakker *et al*., 2017) there is still an ongoing debate to which extent parasite-related changes in host phenotype increases transmission and whether these changes are adaptive for the parasite (Cézilly *et al*., 2010).

According to the *manipulation hypothesis*, parasites that are able to disturb or reverse the anti-predator behavior or cryptic appearance of its intermediate host should benefit from increased predation of the intermediate host (Moore, 2002). However, the evolutionary arms-race between intermediate host and parasites needs not necessarily to be won always by the individual parasite. This argument is supported by the occurrence of population-dependent, differential manipulative abilities of parasites (Franceschi *el al.,* 2010a). Still, studies examining individual behavioral variability of intermediate host are still underrepresented. Instead, parasitic effects are usually examined using average values of behavioral or morphological traits of infected and uninfected host individuals. Such approaches, however, neglect within- and between-individual variation of host responses (Cézilly *et al.,* 2013; Poulin, 2013). As selection requires phenotypic variation at individual level, detailed knowledge about variance components and the factors maintaining variation are crucial in order to gain a comprehensive understanding regarding the evolution of complex host-parasite systems (Thomas *et al.,* 2011). Such variation in manipulative effects might depend on, for instance, parasitic virulence (Alizon *et al.,* 2013), the intensity of infection and inter- as well

as intra-specific interactions between parasites (Mideo, 2009; Cézilly *et al.*, 2014), but also on host resistance (Mazzi and Bakker 2003; Daoust *et al.*, 2015).

Acanthocephalans represent a well-described example of manipulative parasites infecting arthropods as intermediate hosts and vertebrates as final hosts (Kennedy, 2006; Bakker *et al.*, 2017). Infection with an acanthocephalan leads to altered appearance, behavior, physiology and life-history of their intermediate hosts (see Bakker *et al.*, 2017 for a review). Some of these changes are caused by active parasitic manipulation while others are adaptive host responses to resist infection (Cézilly *et al.*, 2010; Bakker *et al.*, 2017). For example, the acanthocephalan *Pomphorhynchus laevis* uses various *Gammarus* species as intermediate hosts and certain fishes as final hosts (Kennedy, 2006). It alters the cryptic appearance of the intermediate host as the conspicuous orange cystacanth (the infective developmental stage of the parasite) is well visible through the cuticle of the gammarid (Kennedy *et al.*, 1978). Such conspicuous color mark makes the intermediate host more prone to predation by three-spined stickleback, *Gasterosteus aculeatus*, a suitable final host for *P. laevis* (Bakker *et al.*, 1997), but not to *Salmo trutta*, representing an unsuitable host for *P. laevis* (Kaldonski *et al.*, 2009). Furthermore, the parasite does not only change the intermediate host's visual appearance, but also its anti-predator behavior. While uninfected *G. pulex* show predator avoidance and are photophobic, individuals infected with *P. laevis* are attracted by predator odor (Baldauf *et al.*, 2007) and show photophilic behavior (Bakker *et al.*, 1997). These behavioral alterations are assumed to increase the probability of predation of the intermediate host, and thus, the transmission of the parasite to the final host (Lagrue *et al.*, 2007). The acanthocephalan *Polymorphus minutus* exploits gammarids as intermediate and water birds as final hosts (Kennedy, 2006). *Polymorphus* species alter the photo- and geotactic behavior of the intermediate host, with infected amphipods being more photophilic and swimming closer to the water surface (Hindsbo, 1972; Bethel and Holmes, 1974; Bailly *et al.*, 2017). Furthermore, they reduce the overall activity of the intermediate host (Thünken *et al.*, 2010).

While such parasite-induced changes are well described on an average population level, individual acanthocephalan-infected amphipods show considerable behavioral variation (Thomas *et al.*, 2011), which can partly be ascribed to differential parasitic effects. For example, modification of intermediate host`s behavior depend on the developmental stage of the parasite. *P. laevis* and *P. minutus* are only infective at the cystacanth stage, but not at the earlier acanthella stage (*P. laevis*: Franceschi *et al.*, 2008; Franceschi *et al.*, 2010b; *P. minutus*: Bailly *et al.*, 2017). Consequently, parasites at different developmental stages have different interests, which are reflected in their manipulative potential (Dianne *et al*., 2010, 2011). While individuals that already reached the infective cystacanth stage shall try to increase predation of the intermediate host by the final host, younger individuals in the acanthella stage are expected to aim at avoiding predation (Hafer and Milinski 2015). Furthermore, there are age-independent sources of manipulative variation. These include season-dependent effects (Benesh *et al.*, 2009; Franceschi *et al.*, 2010b; Bailly *et al*., 2017), as well as genetic differences of individual parasites in the ability to manipulate the intermediate host (Franceschi *et al*., 2010a). Finally, the parasitization intensity, *i.e.* the number of parasites within a single host, affects parasitic manipulation (Cézilly *et al*., 2014). In multiple-infected hosts, cumulated parasitic effects might result in increased manipulation (Franceschi *et al*., 2008). In contrast, competition between individual parasites over limited host resources might impede parasitic growth and development (Cornet, 2011; Dianne et al., 2012), resulting in reduced manipulation (Caddigan *et al*., 2017), especially when manipulation itself is costly (Maure et al., 2013). Finally, parasites at different stages of their life cycle might have opposite interests, which can lead to parasitic effects cancelling each other (*sabotage hypothesis*, Haine *et al*., 2005; Dianne *et al*., 2010; Hafer and Milinski 2015).

Furthermore, differential responses to attempted manipulation by the parasite might be caused by variation of the host individual itself. This variation might occur due to different responses between host individuals or due to high within-individual behavioral inconsistency.

Infection may increase variation between hosts for example when certain individuals are susceptible to infection whereas others are more resistant. Furthermore, infected individuals may be less capable to maintain consistency in behavior, leading to higher within-individual variation compared to uninfected individuals.

Thus far, these different sources of variation in intermediate host responses received only limited attention, despite their importance to fully comprehend parasite-host-coevolution. In the present study we i) describe within- and between-individual behavioral variation in uninfected and infected *G. pulex* and ii) relate intensity of parasitic infection to changes of host behavior. Therefore, we repeatedly tested photophobia and activity in individual gammarids over a period of 17 days. Test animals were either uninfected or carried at least one cystacanth of the manipulative parasite *P. minutus.* To test whether intra-specific competition within a host affects parasitic manipulation, single-infected (no competition for the parasite) or multiple-infected (competition between parasites) *G. pulex* were examined. The competition hypothesis as well as the sabotage hypothesis predict weaker manipulation of *Gammarus*. Alternatively, parasitic effects could add up and, thus, multiple-infected hosts should suffer stronger from manipulation.

Material and Methods

Experimental subjects

Uninfected, single- and multiple-infected *Gammarus pulex* were collected on May 10th 2017 from the brook “Derlebach” in Bonn, Germany (50°42‘N, 7°02’E). At the capture site, the brook measured 50 cm in width and 15 cm in depth. The water temperature was 10°C. Several hundred *G. pulex* were indiscriminately caught using a dip net and pre-sorted into uninfected and infected individuals directly thereafter. Gammarids were transferred to the laboratory using buckets filled with water and decaying leaves taken from the natural habitat. In the laboratory, the infection status of the gammarids was determined visually by checking for the presence and number of the orange cystacanths that were visible through the cuticle of the

dorsal coelom (Bakker *et al*., 1997). Furthermore, gammarids were measured and dissected directly after the experiments. Total length was defined as the distance between the base of the first antenna and the base of the telson, measured to the nearest millimeter with the animal placed on graph paper. Infected and uninfected gammarids did not significantly differ in size (uninfected: 10.38 ± 1.89 mm, mean ± SD; single-infected: 10.07 ± 1.32 mm, mean ± SD; multiple-infected: 9.38 ± 1.26 mm, mean ± SD; Anova, df = 2, F = 1.478, p = 0.242). After the experiment (see below) cystacanths were prepared out of all infected individuals. They were photographed with tenfold magnification using a camera (Hitachi Denshi, HV-C20AMP), attached to a stereomicroscope (Leica, S8AP0). Photos were used to verify parasite species and infection status, i.e. number of parasites and developmental stage. All parasites were cystacanths of *P. minutus.* Their proboscis was completely invaginated and the parasites encased by an envelope and formed ovoidal (Dezfuli *et al*., 2001). The number of parasites in multiple-infected *G. pulex* varied between two to five (2.62 ± 0.26, mean ± SD).

In total 13 uninfected, 13 single- and 13 multiple-infected individuals were separated and kept individually in plastic boxes (18.5 x 11.5 x 13.5 cm, length x width x height) filled with 800 ml of aged tap water. Each box was equipped with an air stone and two gram of decaying leaves, which served as food and shelter. Thus, individuals could choose between bright (open area) and dark (under the leaves) light conditions. About 70 % of the water in each box was replaced once a week with aged tap water. A full spectrum fluorescent tube (True-Light, Natural Daylight 5500, 36W), emitting a spectral emission similar to natural daylight, was placed in a distance of 41 cm above the holding boxes, creating a maximum light intensity of 600 lux (PCE 174 Data logger light meter, PCE instruments). Gammarids were kept at a light–dark cycle of 12L:12D and a temperature of 13 ± 1 °C.

Experimental design

Experiments were conducted between May 11th 2017 and May 27th 2017. Trials were performed on three consecutive days (Tuesday to Thursday) each week, with all individuals

being tested once a day. Thus, each of the 39 gammarids was tested 9 times. For the experiments, two clear plastic tanks, each measuring 24.5 x 15 x 15.5 cm (length x width x height), were placed on a white Styrofoam plate, with the longer sides aligned to each other (Fig. 1). Therefore, two trials could be conducted simultaneously. Tanks were filled with aged tap water to a level of 7 cm. The water temperature of the experimental tanks resembled holding conditions. The long sides of both tanks were covered with grey plastic sheets, so that light could only reach the tank from the short end and from above. The set-up was illuminated by a full spectrum fluorescent tube (True-Light, Natural Daylight 5500, 36W), installed at a distance of 132 cm and at a height of 35 cm above water surface one short side of the set-up. Thus, we created a brightness gradient within each tank (Figure 1). The light intensity in the center of the light-facing half of the respective tank was 39 lux. In the center of the half turned away from the light source light intensity was 31 lux. Above each tank we installed a webcam (Logitech, Webcam Pro 9000) connected to a laptop (Fujitsu Siemens, Lifebook SH531). For each trial, one gammarid was placed within a transparent plastic cylinder (diameter 3 cm) in the middle of each tank. After an acclimation phase of one minute, the cylinders in both tanks were lifted by hand, so that the gammarids were able to swim freely in their tank. Immediately after lifting the cylinders, video recordings were started. A trial lasted 10 minutes. At the end of each trial, gammarids were carefully transferred back to their respective holding boxes. To exclude potential side effects, the direction of the light source was switched after every fifths trial.

Motion analyses

Video recording were analyzed using the tracking software Biobserve Viewer III (Biobserve GmbH, version 3.0.0.119). The test tank was virtually divided into two equal sized zones, one facing the light source (light) and the other one the opposing side (dark). The *Gammarus* was continuously tracked for the experimental phase of 10 minutes. Time spent in each zone and changes between zones were determined and exported to Microsoft Excel. A phototaxis index

was calculated (time on light side – time on dark side). Activity was estimated by the number of changes between the light and dark side.

Statistical analyses

Statistical analyses were conducted in R, version 3.42 (R-Development-Core-Team, 2013). When data deviated from normality they were Box-Cox-transformed or non-parametric test were applied. Between-individual differences across and within infection groups (uninfected, single-infected and multiple-infected) in phototaxis and activity were examined by fitting linear models (lm) with individual gammarid as explanatory variable. To test for between-individual behavioral variation among infections groups we first calculated mean values for each gammarid and then compared variation among infections groups with Levene-tests. To compare within-individual variation we first calculated a coefficient of variation (the ratio of the standard deviation to the mean) for each gammarid and then compared infection groups using Kruskal-Wallis rank sum tests. Repeatability was calculated with the R package "rptR" (see Stoffel *et al.*, 2017). To examine behavioral differences among infection groups we applied linear mixed effect models (lme using the R package “nlme”) with activity or phototaxis as dependent variables, infection group as explanatory factors and individual gammarid as random factor. We added experimental day as covariates to the model to examine whether phototaxis or activity changed over the course of the experiment and whether this relationship differed between infection groups (day x infection group interaction). Within infected gammarids, we investigated the effect of intensity of parasitization (number of parasites within a host) on phototaxis and activity, respectively, fitting linear models. The relationship between phototaxis and activity was examined using a lm with phototaxis (based on mean value, see above) as response variable and activity as explanatory variable. To test for differences in the relationship between phototaxis and activity between infection groups we included the activity x infection group as interaction term into the model. All non-significant interaction terms were removed from the models

(Engqvist 2005). All tests were two-tailed, alpha values smaller than 0.05 were considered as being statistically significant.

Results

Phototaxis

Individual gammarids varied in phototaxis across infection groups (lm, $\Delta df = 38$, $F = 8.498$, $p < 0.001$, Fig. 2a) as well as within groups (uninfected: lm, $\Delta df = 12$, $F = 2.973$, $p = 0.001$, single-infected: $\Delta df = 12$, $F = 6.157$, $p < 0.001$; multiple-infected: $\Delta df = 12$, $F = 8.503$, $p < 0.001$). Infection status influenced phototaxis (Table 1). On average, single-infected gammarids behaved randomly concerning phototaxis (one sample t-test, $t = -0.723$, $\Delta df = 12$, $p = 0.483$, Fig. 2b) and differed in phototaxis from multiple-infected gammarids and uninfected ones (lme, both $\Delta df = 1$, both $\chi^2 > 3.897$, both $p < 0.05$, Fig. 2b). Multiple- and uninfected gammarids did not differ significantly from each other (lme, $\Delta df = 1$, $\chi^2 = 2.744$, $p = 0.100$, Fig. 2b) and both groups were on average photophobic (one sample t-tests, both $\Delta df = 12$, both $t < -3.420$, both $p < 0.01$, Fig. 2b). Individual variation in phototaxis differed between infection groups (Levene-test, $\Delta df = 2$, $F = 4.142$, $p = 0.024$, Fig. 2a), with single- and multiple-infected gammarids being more variable than uninfected ones (Levene-tests, both $\Delta df = 1$, both $F > 5.800$, both $p < 0.025$, Fig. 2a). Single- and multiple-infected gammarids did not differ significantly in this respect (Levene-test, $\Delta df = 1$, $F = 0.119$, $p = 0.732$, Fig. 2a). All uninfected gammarids avoided the illuminated side, whereas in infected gammarids, photophobic and photophilic individuals were present as well as those behaving randomly concerning light response. During the course of the experiment (17 days), phototaxis did not change significantly (Table 1). Within individual variation did not differ significantly between infection groups (Kruskal-Wallis rank sum test, $\Delta df = 2$, $\chi^2 = 0.560$, $p = 0.755$, see also table 2 for repeatability values). Within infected gammarids, number of parasites did not significantly affect phototaxis (lme, $\Delta df = 1$, $\chi^2 = 0.020$, $p = 0.886$).

Activity

Individual gammarids varied in activity across infection groups (lm, $\Delta df = 38$, $F = 8.467$, $p < 0.001$, Fig. 3a) as well as within each group (uninfected: lm, $\Delta df = 12$, $F = 3.072$, $p = 0.001$, single-infected: $\Delta df = 12$, $F = 10.886$, $p < 0.001$; multiple-infected: $\Delta df = 12$, $F = 9.612$, $p < 0.001$). Infection status affected activity (Table 1). On average, infected gammarids (single- and multiple-infected individuals did not differ significantly from each other (lme, $\Delta df = 2$, $\chi^2 = 0.276$, $p = 0.599$), and were less active than uninfected individuals (lme, both $\Delta df = 2$, both $\chi^2 > 5.517$, both $p < 0.02$, Fig. 3b). Between-individual variation did not significantly differ between infected and uninfected gammarids (Levene-test, $\Delta df = 2$, $F = 1.124$, $p = 0.336$, Fig. 3a). Individual coefficient of variation did not differ significantly between infection groups (Kruskal-Wallis rank sum test, $\Delta df = 2$, $\chi^2 = 0.560$, $p = 0.755$, see also table 2 for repeatability values). In infected gammarids, number of parasites did not significantly affect host`s activity (lme, $\Delta df = 1$, $\chi^2 = 1.854$, $p = 0.173$).

Relationship between phototaxis and activity

Phototaxis did not significantly correlate with activity (lm, $\Delta df = 1$, $F < 0.001$, $p = 0.984$). This effect was similar in infection groups (activity x infection group interaction: lm, $\Delta df = 1$, $F = 0.361$, $p = 0.699$).

Discussion

Understanding individual behavioral variation is a classical topic in evolutionary and behavioral ecology research (Bakker, 1986; Bell *et al.*, 2010) and has regained considerable attention recently in the framework of animal personality (Barber and Dingemanse, 2010; Beekmann and Jordan, 2017). However, individual variation in parasite-host interaction has been considered to greater extent only recently (Thomas *et al.*, 2011; Poulin, 2013).

In our study, both uninfected and *P. minutus*-infected gammarids showed repeatable individual differences in phototaxis and activity. Repeatabilities ranged from approximately

20% to 50% and were thus similar to those found in other behavioral traits in a range of animal taxa (Bell *et al*., 2010). Repeatability values for infected gammarids were higher than those of uninfected ones. This probably resulted from higher between-individual variation in infected gammarids compared to uninfected ones, as indicated by similar coefficients of variation between infection groups. In line with these findings, Benesh *et al*., (2008) found repeatable activity in isopods infected with *Acanthocephalus lucii,* but not in uninfected ones. In contrast, Coats *et al*. (2009) report higher repeatability in uninfected amphipods compared to infected conspecifics. The contrasting results may reflect differences among species in manipulative capabilities of parasites or host resistances (Franceschi *et al.*, 2010; Thomas *et al.*, 2011, see below).

Phototaxis

Between-individual variation in phototaxis was greater between infected individuals. While uninfected gammarids were uniformly photophobic (indicating strong selection on photophobia), infected individuals showed the full behavioral range from photophobia to photophilia.

The high variation observed in single-infected individuals might be explained by some cystacanths having not yet reached the manipulative stage. Indeed, even at cystacanth stage it takes further maturation or establishment within the host until manipulation becomes apparent (Bethel and Holmes, 1974; Dianne *et al.*, 2010). Consequently, young cystacanths of *P. minutus* and *P. laevis* are less manipulative than older ones (Franceschi *et al.*, 2008; Bailly *et al.*, 2017). Bethel and Holmes (1974) showed that cystacanths of the closely related *Polymorphus paradoxus* induce alterations in the host only 17 days after reaching that stage. As we used naturally infected gammarids we do not have information about the exact age of the parasite. However, if the described variation was caused by age effects one would expect photophilia to increase over the course of the experiment in infected gammarids, as cystacanths aged during this time as well. As we did not find any significant time effects, high

variation in manipulation most likely did not result from age differences between cystacanth. Rather, it might either depend on the host's ability to resist manipulation, on individual parasites manipulative abilities, or a combination of both. Indeed, it has been shown that sibships of the manipulative acanthocephalan *P. laevis* differ in manipulative abilities (Cornet *et al.*, 2009; Dianne *et al.*, 2012) and that gammarid hosts can develop resistance against local manipulative parasites (Franceschi *et al.*, 2010a).

Interestingly, mean photophobic responses of multiple-infected gammarids were comparable to those of their uninfected conspecifics. Thus, parasite's effects did not add up, not leading to a stronger response. In contrast, our results suggest that intra-specific competition among parasites dampens their manipulative effects. This effect can be explained in two ways. First, intra-specific competition within the host might have affected the parasite development (Dezfuli *et al.*, 2001; Franceschi *et al.*, 2008; Dianne *et al.*, 2010). Provided that host resources are limited, and manipulation is costly, cystacanths sharing a host may need longer to reach maximum manipulative potential. Second, parasites at different developmental stages will have different manipulative interests. While older, highly infective cystacanths aim at getting predated by a bird, younger ones would favor to remain in the intermediate host for longer. Thus, lower photophilic behavior might be explained by cystacanths actively competing over control of their *Gammarus* host.

Activity

In line with earlier studies (e.g. Thünken *et al.*, 2010) infected gammarids were less active compared to their uninfected conspecifics. Interestingly, multiple-infected individuals showed similar activity than single-infected gammarids and the number of parasites within a gammarid was not significantly correlated with activity. This suggests that the additional load by the parasite is not responsible for the changes in host's activity. Although infected gammarids were on average less active, a proportion of infected individuals showed a similar activity as uninfected ones (cf. Fig. 3a), suggesting that specific individuals only respond with

changes in activity or that reduced activity is only present at a specific time point, e.g. when the parasite actively interferes with the physiology of the host. Furthermore, in contrast to phototaxis, variation among individuals in activity was similar between infection groups, supporting the findings of earlier studies that changes in activity are side-effects of the infection rather than the result of active manipulation (e.g. Poulin, 1998; Thünken *et al*., 2010). Future research should address these questions in more detail.

Another source of individual variation within infection groups might be the sex of the gammarids. Indeed, acanthocephalan parasites reduce female fecundity (Bollache *et al*., 2002). However, evidence for sex-specific behavioral responses to infection is ambiguous. Park and Sparkes (2017) found that *Acanthocephalus dirus*-infected males and females of *Caecidotea intermedius* differ in refuge use while Bailly *et al*. (2017) did not find sex-specific phototactic responses of *P. minutus*-infected gammarids. We did not explicitly determine the sex of the gammarids used in our study. However, animals of the different infection groups were similar in size. Given the size-range of the used animals suggests that we used both male and female *G. pulex* (Adams and Greenwood, 1983). Therefore, the differences between the three different groups cannot be explained by sex differences. However, the variability within the infected groups might be caused by different reactions of infected males and females. This hypothesis might be investigated in more detail in future studies.

In summary, we show that individual gammarids differ in their risk-adverse behavior. Furthermore, we demonstrate high variation in manipulative success of an acanthocephalan parasite, which could be explained by between-parasite competition within an intermediate host and differential responses of individual hosts to manipulation. Such variation in responsiveness underlines the ongoing arms-race between the parasite and its host and shed light on the evolution of trophical transmitted parasites and their hosts.

Author contributions

TT, SV, and SAB conceived the study. SV and TJ conducted the experiments. TT analyzed the data. TT, SV and JGF discussed the results. TT, SV and JGF wrote the manuscript. The final draft was approved by all authors.

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Figures:

Figure 1

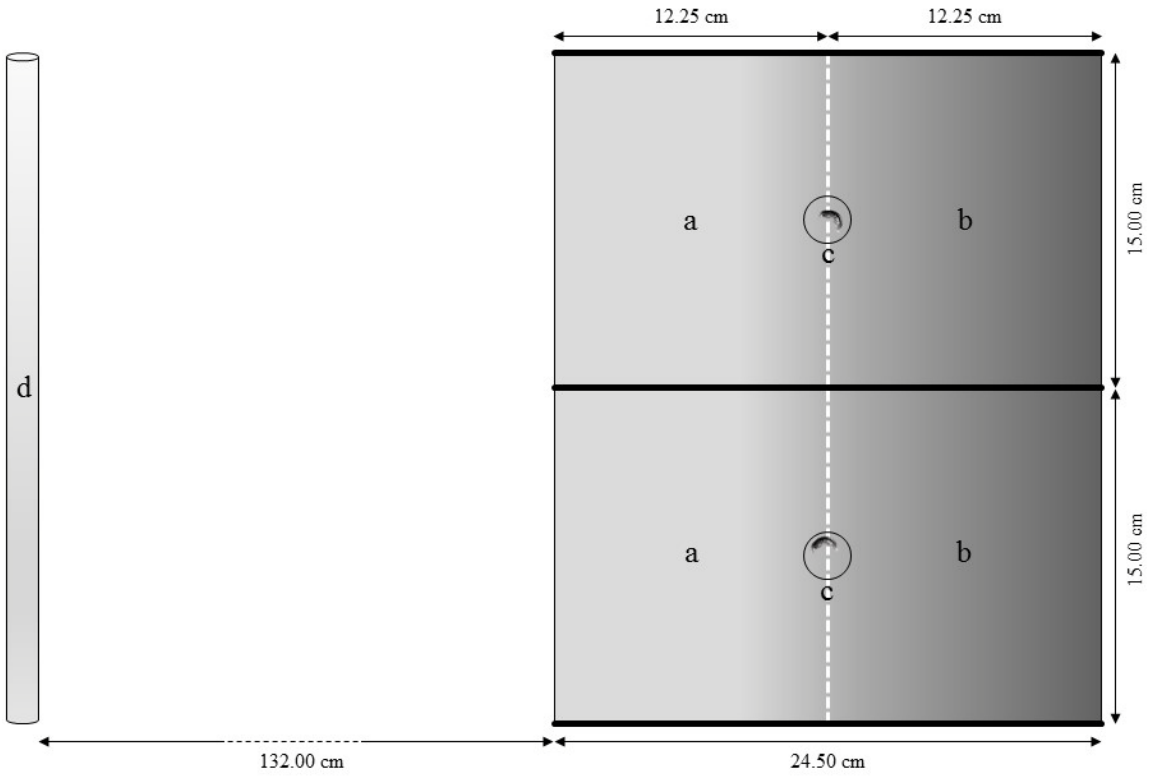
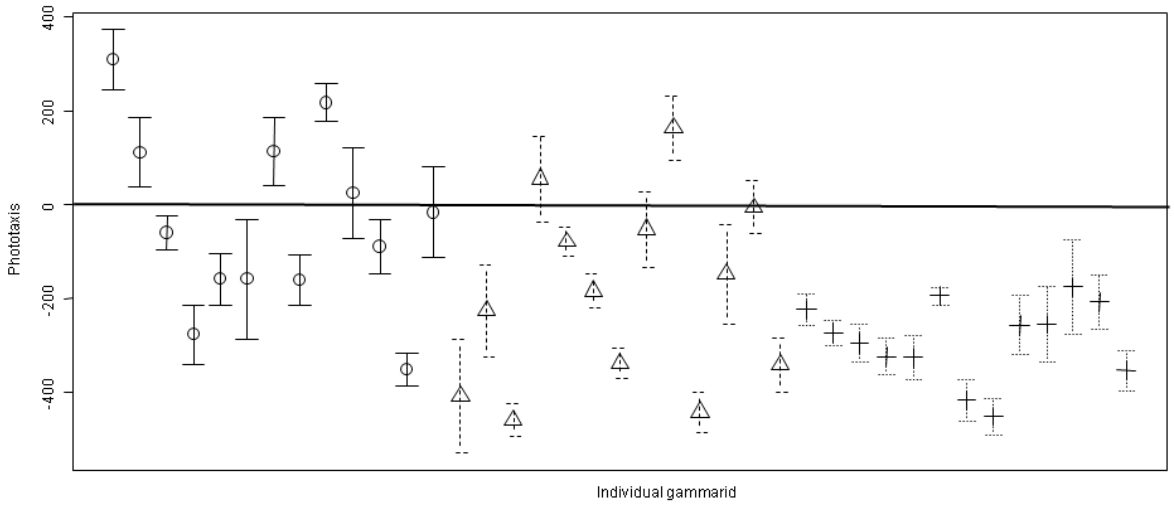


Fig. 1: Schematic figure of the experimental set-up. Two plastic tanks were placed alongside to each other and visually separated. A brightness gradient was created by placing a slightly elevated light source (d) 132 cm away from one side of the set-up. For tracking-software analyses, two virtual zones were created with one facing the light source (a) and an opposing one (b). The transparent cylinder (c) was lifted after one-minute acclimation time.

Figure 2

a)



b)

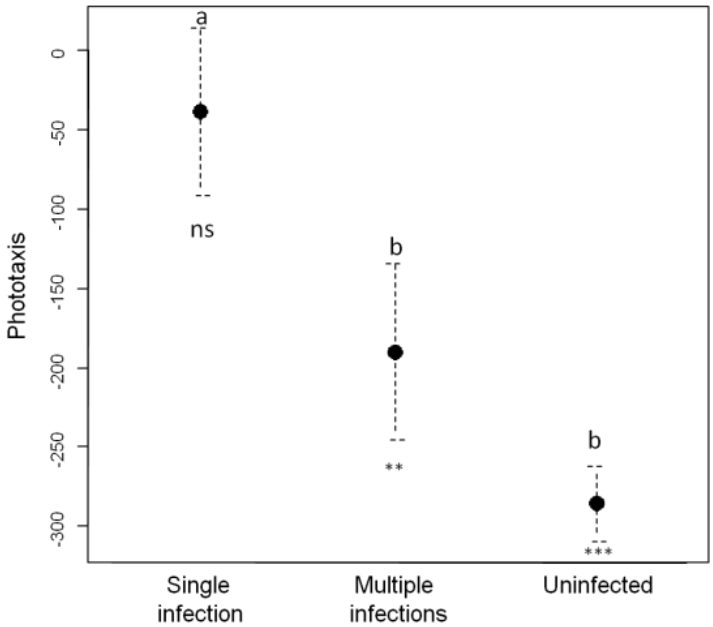


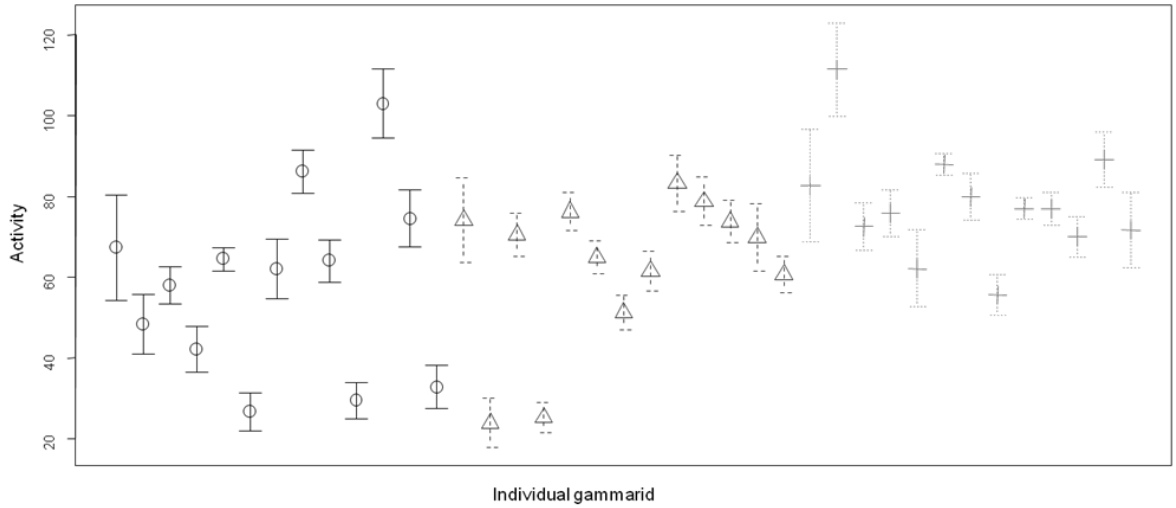
Fig. 2

a): Phototaxis scores (time on light side minus time on dark side; values > 0 photophilic, values < 0 photophobic) for individual gammarids: single-infected (circles), multiple- infected (≥2 parasites, triangles), uninfected (crosses). Shown are mean values and SE for each tested individual.

b): Phototaxis mean values and SE for the infection groups. Different letters above means indicate significant difference between groups ($p < 0.05$). Symbols below means indicate significant deviation from 0 (ns, $p > 0.05$, **; $p < 0.01$, ***; $p < 0.001$).

Figure 3

a)



b)

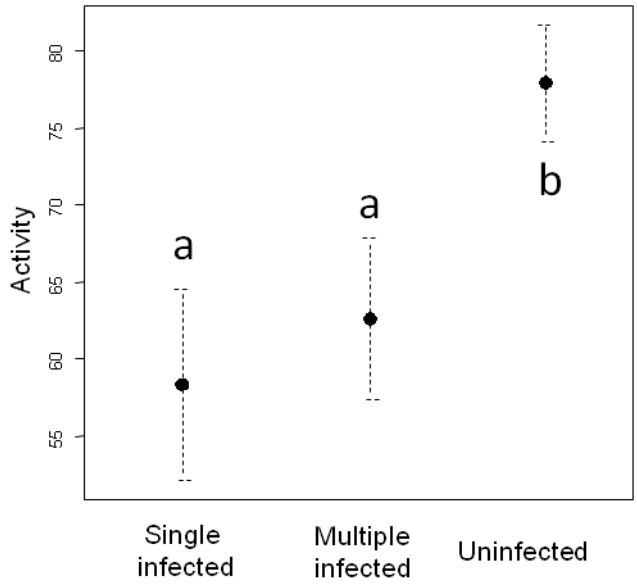


Fig 3:

a) Activity, i.e. zone changes, for individual gammarids: Single-infected (circles), multiple-infected (≥2 parasites, triangles), uninfected (crosses). Shown are mean values and SE for each tested individual.

b) Activity mean values and SE for the infection groups. Different letters above means indicate significant difference between groups ($p<0.05$).

Tables

Table 1: Results of linear mixed effect models (with individual as random factor). Effects of infection (uninfected, single-infected and multiple-infected) and experimental day (day) on gammarids' phototaxis and activity.

dependent variable	interaction / fixed factor	N	Δdf	χ^2	P
Phototaxis	Infection group	39	2	11.732	**0.002**
	Infection group x days	39	2	0.712	0.700
	Days	39	1	3.058	0.080
Activity	Infection group	39	1	7.609	**0.022**
	Infection group x days	39	2	3.958	0.138
	Days	39	1	6.721	**0.009**

Table 2: Repeatability (R) with standard error (SE), 95% confidence intervals (CI) and p-values for phototaxis and activity for each infection group.

Variable	Infection group	R	SE	CI	P
Phototaxis	Uninfected	0.192	0.098	0.006, 0.401	**0.003**
	Single-infected	0.382	0.118	0.127, 0.593	**<0.001**
	Multiple-infected	0.460	0.122	0.185, 0.647	**<0.001**
Activity	Uninfected	0.198	0.101	0.020, 0.406	**0.002**
	Single-infected	0.528	0.121	0.238, 0.710	**<0.001**
	Multiple-infected	0.496	0.126	0.195, 0.694	**<0.001**